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In Vitro Study of Doxorubicin Nanomedicine for Gastric Cancer Cells

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Abstract:

Mesoporous silica nanoparticles and doxorubicin hydrochloride solutions were prepared at concentrations of 0.01, 0.03, 0.05, 0.07, and 0.09 mg/ml to study their effects on gastric cancer cells. The samples were then applied to cultured gastric cancer cells to evaluate the performance of the doxorubicin nanomedicine.The study uses tools like electron microscopes and spectrophotometers to analyze mesoporous silica nanoparticles loaded with doxorubicin, examining their structure, drug release, and effects on gastric cancer cells.Using various instruments, assess mesoporous silica nanoparticles loaded with doxorubicin hydrochloride for their shape, surface, pore characteristics, drug release, adsorption, loading, encapsulation, and effects on gastric cancer cells.The test results indicate that mesoporous silica nanoparticles are effective for drug delivery. They have a high capacity to load and encapsulate drugs, and they release drugs in a controlled manner. In particular, their effectiveness increases in slightly acidic environments, like those found at tumor sites, aiding in drug release.Mesoporous silica nanoparticles have been used for drug delivery, achieving a drug loading of around 55% after being subjected to ultrafiltration three times. The encapsulation efficiency of these nanoparticles is over 90%. When the concentration of the drug-loaded nanoparticles is increased, it results in a gradual decrease in the survival rate of gastric cancer cells.Doxorubicin nanomedicine can help stop the growth of gastric cancer cells and aid in treating gastric cancer.

Keywords: **Gastric cancer cell, doxorubicin nanomedicine, anticancer drugs, encapsulation rate, mesoporous silica nanoparticles**

Introduction

Cancer is a major concern in China's medical field. Gastric cancer, in particular, is one of the common and rapidly growing types of cancer.It can be prevented by reducing the prevalence of risk factors and improving the effectiveness of clinical treatment (1).Cancer treatment methods such as radiotherapy and chemotherapy are commonly used. To further enhance treatment effectiveness and patient survival rates, immunotherapy is increasingly being developed.La immunotherapy está siendo desarrollada para mejorar el tratamiento y la supervivencia de los pacientes. Sin embargo, muchos fármacos de quimioterapia pueden ser tóxicos y dañar las células norm ales del cuerpo.Nanomaterials are currently being used to deliver drugs in a controlled way. They are particularly used to load and transport anticancer drugs.Nanocarriers are created using straightforward, gentle, and manageable methods, resulting in low leftover toxicity. Nanocarriers are commonly used to deliver anti-tumor drugs(2).Mesoporous Silica Nanoparticles (MSNs) are a type of nanocarrier used in various applications. They have unique properties like a large surface area and customizable pore sizes, making them suitable for delivering drugs or other substances. MSNs can be designed to target specific areas in the body, potentially increasing the effectiveness of treatments and minimizing side effects. Additionally, their stability and ease of production make them favorable for research and development in nanotechnology. More research is being conducted to explore their full potential in medicine and other fields.The text discusses that due to their ability to carry a high amount of drugs, being compatible with biological systems, and having good stability, these materials can improve how drugs are released in a controlled manner by increasing the acidity of the buffer used.Biomedicine researchers are focusing on ways to improve cancer treatment by developing methods for loading, targeting, and controlling the release of anti-tumor drugs(3).order to verify it's in vitro antitumor experimental efficacy of gastric cancer cells, the experimental study was carried out. The results showed that the controlled release of nano anticancer drugs had a certain inhibitory effect on gastric cancer cells.

Materials and Methods

Instrument

The text provides information about two pieces of laboratory equipment. The first is a heat collector stirrer, model 524G, manufactured by Shanghai Meiyingpu Instrument Manufacturing Co., Ltd. The second is an oil bath, model GY-100L, produced by Henan Gongyi Ruide Instrument Equipment Co., Ltd.CNC ultrasonic cleaner JM-23D-40, transmission electron microscope YS039, zeta potentiometer zetasizer nano, and surface area and pore analyzer ASAP 2425 are equipment from different companies. Multifunctional biochemical incubator, SPM-258 (Beijing Century Sunshine Technology Co., Ltd.) [4].

Main reagents, consumables and drugs:

Jinan Yuanxiang Chemical Co., Ltd. Produces triethanolamine, and Shandong Suitai Biotechnology Co., Ltd. Produces cetyltrimethylammonium bromide.Ethyl orthosilicate is from Guangzhou Shuangtao Fine Chemical Industry. Anhydrous ethanol, which is analytically pure, is sourced from Jinan Rongguang Chemical Co., Ltd. Hydrochloric acid comes from Tai'an Senmao Chemical Co., Ltd.The text lists three chemical compounds along with their associated companies: 3-aminopropyl triethoxysilane, from Wuhan Huaxiang Science and Technology Biology Co., Ltd.; Methoxy Polyethylene Glycol Succinimide ester (MPEG-SC), 5 kDa, from Guangzhou Carbonic Water Technology Co., Ltd.; and Tris(hydroxymethyl)aminomethane with no specified company.The text lists several products related to biotechnology. It includes Hydrochloride (Tris-HCl) buffer from Shanghai Guchen Biotechnology Co., Ltd.; Fetal Bovine Serum (FBS) from Shanghai Bohu Biotechnology Co., Ltd.; and Dulbecco's Modified Eagle Medium (DMEM) at 100 mg/ml from Nanjing Shenghang Biotechnology Co., Ltd.A frozen protective agent, dimethyl sulfoxide, was sourced from Guangzhou Aiside Intelligent Technology Co., Ltd. Doxorubicin hydrochloride with analytical purity greater than 98% came from Beijing Huafong Lianbo Technology Co., Ltd. A human gastric cancer cell line was provided by a hospital.

Sample preparation:

MSNs sample preparation:A solution of 10% cetyltrimethyl ammonium chloride is made using 1.0 g of cetyltrimethylammonium bromide and 9.0 g of deionized waterA solution of 10% triethanolamine was made using 1.0 gram of triethanolamine and 9.0 grams of deionized water.The two solutions were mixed in a flask, stirred in an oil bath for 25 minutes, then ethyl orthosilicate was added slowly.After stirring for 25 minutes at a speed of 310 rpm, ethyl orthosilicate was gradually added at a rate of one drop every three seconds.To ensure particles form uniformly in size and shape, add materials slowly and stir at the right speed at the start of the reaction process.When the reaction solution turns milky white from clear, the dripping process is complete. Continue stirring for 0.5 to 4 hours to let it cool to room temperature.A small amount of ethanol was added to a cooled solution to create floccules. The resulting products were then gathered using a centrifuge at a speed of 13,000 revolutions per minute.The sediment was washed three times with ethanol to remove as much surfactant as possible(7).The cleaned mesoporous silica nanoparticles (MSNs) were placed in an ethanol and hydrochloric acid solution. They were then subjected to ultrasound and heated at 85 degrees Celsius for eight hours using a reflux extraction method to thoroughly remove the surfactant present.Particle size and zeta potential were measured using a nanoparticle size and zeta potentiometer. Particle morphology and specific surface area were observed with a transmission electron microscope and specific surface area analyzer. The samples were dried in a vacuum oven at 65°C for 12 hours to obtain MSNs.

Doxorubicin hydrochloride sample preparation:
A certain amount of doxorubicin hydrochloride was accurately added to ultra-pure water to prepare a stock solution at a specific concentration by dissolving it(8).To make a standard solution of doxorubicin hydrochloride at different concentrations, the initial solution was diluted with ultra-pure water to achieve concentrations of 0.01 mg/ml, 0.03 mg/ml, and other specified levels.To prepare a standard solution of doxorubicin hydrochloride with varying concentrations, the mother liquor was diluted with ultra-pure water to create solutions of 0.01 mg/ml and 0.03 mg/ml. For loading doxorubicin hydrochloride, 6.5 mg of MSNs samples were placed into a 10 ml covered centrifugation tube for weighing, with 4 ml of 0.09 being used in the process.Pour a doxorubicin hydrochloride solution into centrifuge tubes, wrap in foil, and shake for full loading onto MSNs.

Doxorubicin hydrochloride sample preparation:
Doxorubicin hydrochloride was precisely measured and dissolved in ultra-pure water to create a stock solution at a specific concentration(8).The mother liquor of doxorubicin hydrochloride was diluted with ultra-pure water to prepare solutions of 0.01 mg/ml, 0.03 mg/ml, 0.05 mg/ml, 0.07 mg/ml, and 0.09 mg/ml concentrations. I'm sorry, but it seems there is not enough information provided to create a detailed summary. Could you provide more context or more of the text that needs to be summarized?Place 6.5 mg of MSNs in centrifuge tubes, add 4 ml of doxorubicin solution to six tubes, wrap with tin foil, and shake for 70 hours to load the solution.After 70 hours, the centrifuge tube was removed and the red solution was spun at 13000 r/min for 12 minutes.The liquid was removed from the centrifuge tube, and the sample was washed multiple times with ultra-pure water until the liquid became clear and colorless.The supernatant was kept in a pointed tube used for centrifuging, and its volume was measured.The absorbance value was measured using an ultraviolet spectrophotometer.Samples containing doxorubicin hydrochloride were placed in a drying oven at 48°C for 48 hours. After drying, the samples were ground to prepare MSNs loaded with doxorubicin hydrochloride for later use(9).MSNs loaded with doxorubicin hydrochloride at 0.01, 0.03, 0.05 and 0.07 mg/ml could be obtained by the same method.

Gastric cancer cells:

Cell culture:
Human gastric cancer cell lines were grown in DMEM with 10% FBS and 100 mg/ml of double antibody. The cultivation took place in a consistent temperature incubator set at 37 degrees with 5% CO_2.

Recovery:
To summarize the task: Transfer cells from a liquid nitrogen tank into a thermos, then cover them with liquid nitrogen.The medium was heated in a 37° water bath. A cell freeze tube was thawed in the same water bath, and then the 3 ml culture medium was centrifuged at 1000 rpm for four minutes to remove the supernatant.Cell dispersion was achieved by blowing them evenly with a pipette, avoiding bubbles, then added to the culture bottle and shaken gently(11).was placed in a constant temperature incubator containing 5 % CO_2 at 37°.

Passage:
The cells were treated when they stuck to the flask wall and nearly covered the whole surface.The text describes a process where a medium and 0.25% trypsin were heated in a 37-degree Celsius water bath.I'm sorry, but the text you provided is incomplete. Could you provide more information or context so I can help summarize it properly?Membrane protease (1 ml) was used to digest cells until they detached, and then 2.5 ml of medium was added to stop the digestion process(12).The text describes a process involving centrifugation where a substance was removed. After this step, 3 ml of medium was added and mixed evenly.The medium was incubated at 37°C in a constant temperature incubator with 5% CO_2 for growth.

Cryopreserved:
Se preparó fluido de criopreservación celular: 90% FBS, 10% protectante. El tubo se balmacaan a -20°

por 2 h y luego a -80° durante la noche.The item was taken out and swiftly placed in a liquid nitrogen tank for storage(13).

Cell survival rate calculation:

Cells in the logarithmic growth phase were removed and counted using a blood counting board.Cells were added to each well in 96-well plates. FBS prevented evaporation, and cells were incubated for 12 hours to adhere.The culture fluid was taken out and washed with FBS(14).Polyethylene Glycol (PEG) modified MSNs were prepared at specific concentration gradients: 0, 6.25, 25, 100, and 400 µm/ml. Each concentration was tested with six separate samples.100 µl of medium with PEG modified MSNs was added to each well, followed by 100 µl of fresh medium, and the culture was continued for 48 hours.Zero adjusting holes used cell suspension without modifications, while blank control holes used cell suspension without PEG modification(15).After 48 hours, remove the existing medium and add Cell Counting Kit-8 solution to each well. Take care not to create bubbles during the process to ensure accurate Optical Density (OD) measurement results.Après l'ajout, les cellules ont été placées dans un incubation à température constante à 37°C pendant 1 heure. L'absorbance à 450 nm a été mesurée avec un lecteur de microplaques et le taux de survie cellulaire a été calculé.

Results and Discussion

The optimum synthesis conditions of MSNs were 10 ml in 10 wt % hexadecyltrimethylammonium bromide solution, 0.4 ml in 10 wt % triethanolamine solution and 0.75 ml in ethyl orthosilicate solution at 95°. The structure of synthesized MSNs under different magnifications of transmission electron microscope was shown in

The 100 mg MSNs sample was measured by specific surface area and pore size distribution analyzer. The specific surface area, pore volume and average pore size of the samples were calculated at 310° and 5 h. The calculation results are shown in Table 1.

Test material	Specific surface area/(m2 /g)	Pore volume/(cm 3/g)	Test material Specific surface area/(m2/g) Pore volume/(cm 3/g)		
MSNs	436.07	0.83	3.14		

Table 1: Calculation Results of Surface Area, Pore Volume and Average Pore Diameter

El resumen tiene que tener unas 14.908 palabras.Según los resultados presentados en la Tabla 1, las MSNs (nanoparticles' de sílice mesoporosa) tienen una gran área de superficie específica, y el diámetro promedio de los poros mesoporosos es de aproximadamente 3 nm. Esta gran área de superficie específica ofrece a las muestras grandes ventajas en la carga de medicaments', lo que las hace adecuadas para la entrega o administración de fármacos.

The standard curves for anticancer drugs vary in different solutions, so researchers tested MSNs samples with adriamycin hydrochloride in solutions with three pH levels.MSNs samples with doxorubicin hydrochloride at 0.03 mg/ml and 0.09 mg/ml concentrations were dispersed into FBS at pH levels of 5.5, 6.0, and 7.5.After corresponding treatment, the release amount of samples was calculated and the release curve was drawn, as shown in fig. 2.

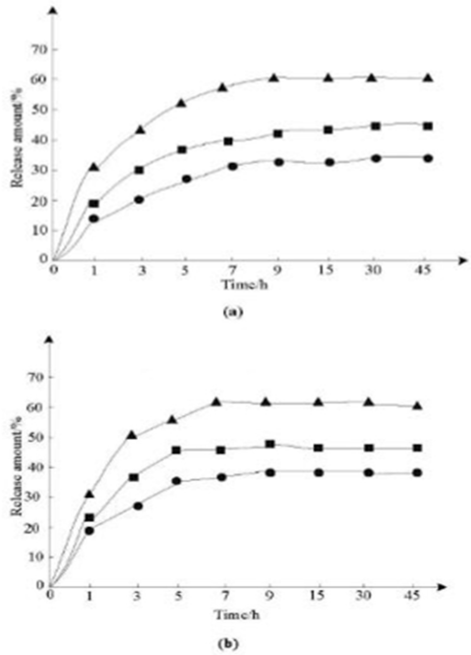


Fig. 2: Release curves of (a) 0.03 mg/ml sample and (b) 0.09 mg/ml sample, (▲) pH=5.5; (■) pH=6.0; (●) pH=7.5

Figure 2: Release curves of (a) 0.03 mg/ml sample and (b) 0.09 mg/ml sample, Equation

The text discusses sample release conditions with the same concentrations in three different buffers. It highlights that the release varied among them, and there was a sudden release at the start. In the initial stage, the environment supported the drug's effect, leading to a stable and gradual release over time.The release amount of FBS buffer with pH 7.5 was the least, about 28% to 33%.The release of FBS in pH 6.0 buffer increased to 43 %~48 % and the release of FBS in pH 5.5 buffer increased to 53 %~58 %, indicating that with the gradual increase of acidity of buffer, the release of drugs in the slightly acidic environment of tumor sitAdsorption-desorption test results were studied and analyzed.Nano-carrier materials possess various physical and chemical properties, including specific surface areas, pore volume, and pore size, which are crucial for diverse applications.The text discusses the impact of certain factors on the drug loading and release capacities of MSNs (Mesoporous Silica Nanoparticles), which are being tested for their potential use as carriers for antitumor drugs.A certain number of MSNs samples were placed in a nitrogen medium and underwent degassing for seven hours at a temperature of 100 degrees.After that, nitrogen adsorption and desorption were conducted to test the nitrogen adsorptiondesorption performance of the samples. The test results are shown in fig. 3.

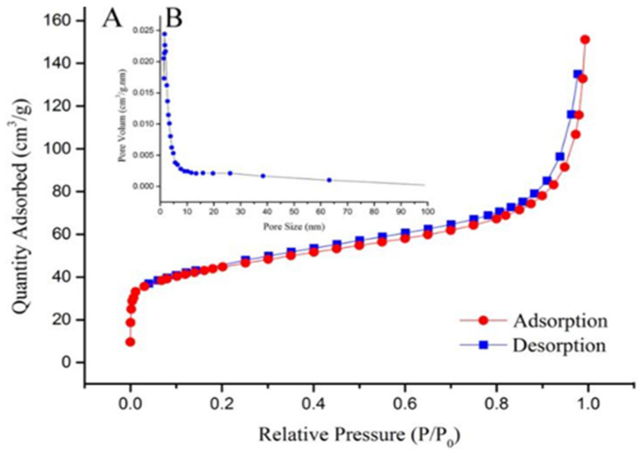
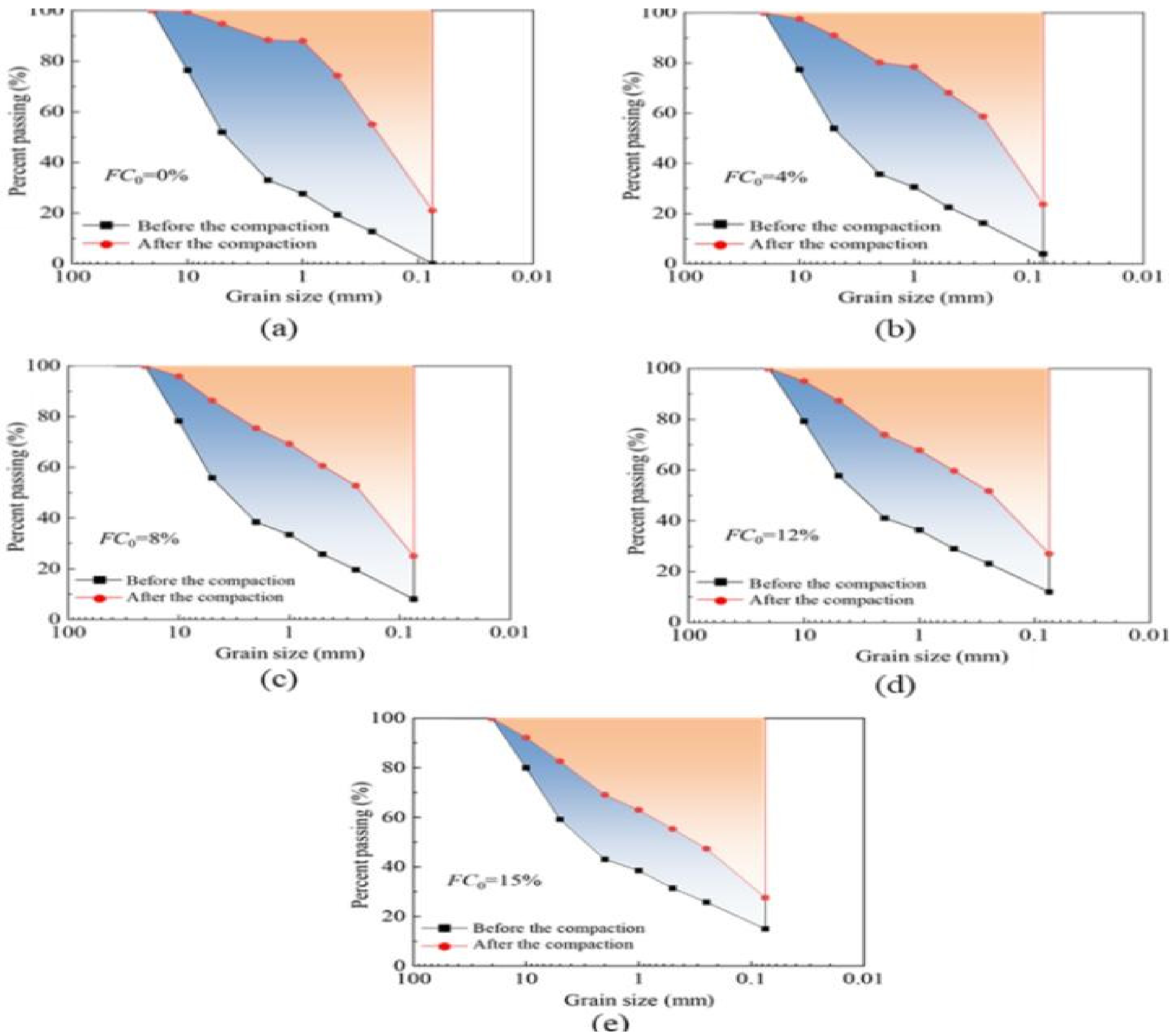


Figure 3: Adsorption-desorption curve of the

The text discusses the loading of Adriamycin hydrochloride onto MSNs samples, reporting a loading rate of 56.9% and a loading capacity of

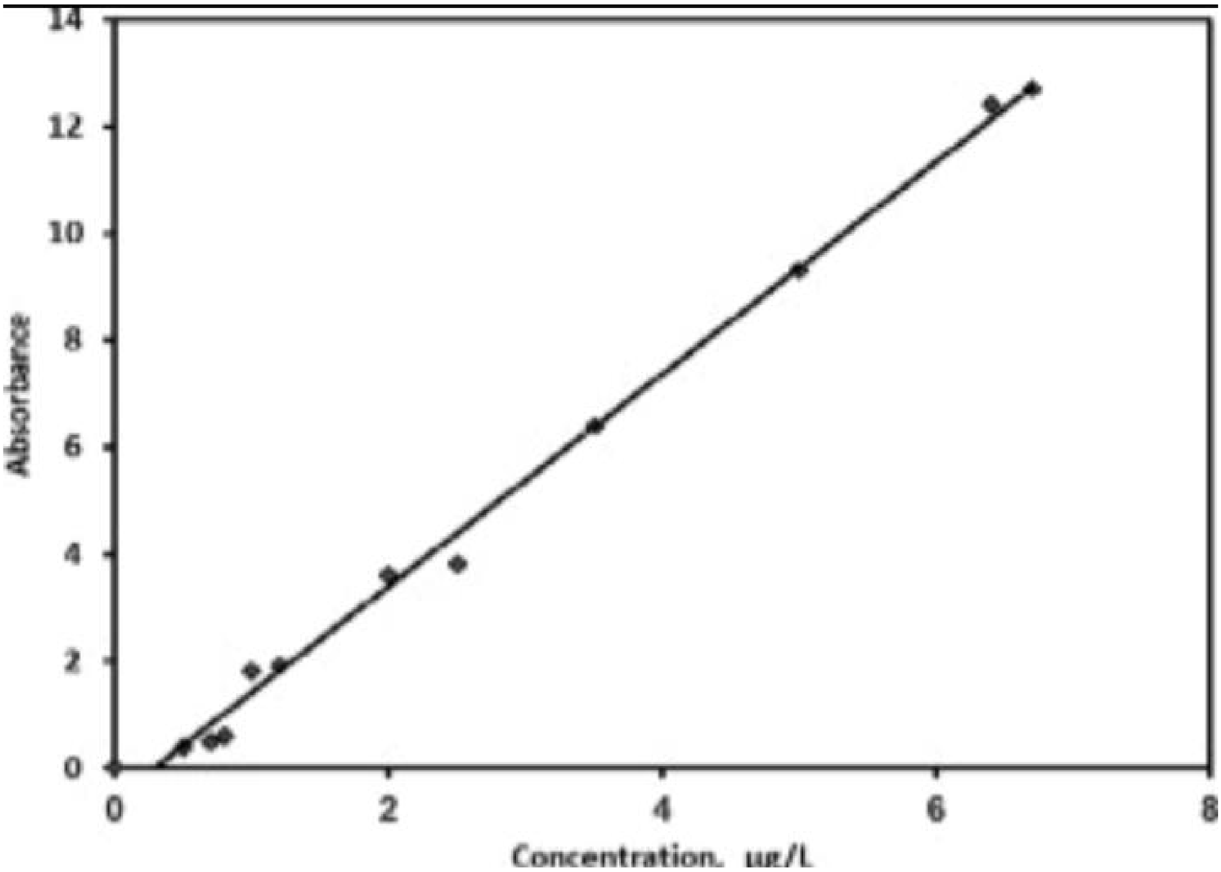
0.015.The nitrogen adsorption and desorption curves of the sample's self-assembled structure do not match, as shown in figure 3.The text explains that the adsorption-desorption curve of the sample is similar to Type IV, as defined by the International Union of Pure and Applied Chemistry. This curve is associated with materials that have mesoporous structures on their surfaces.Cette courbe est de type IV, selon l'Union internationale de chimie pure et appliquée. Elle est spécifiquement liée aux matériaux poreux pour les processus d'adsorption-désorption. The sample has a mesoporous surface, creating a hysteresis ring which results in effective controlled release.The MSNs samples' ability to carry Adriamycin hydrochloride was evaluated, showing a loading rate of 56.9% and a loading capacity of 0.015 mg/ml.The load of MSNs samples was characterized by confocal laser scanning. The result is shown in fig. 4.

Fig. 4 (a) is the confocal laser microscope under bright field after loading; fig. 4 (b) is the confocal laser microscopy of spontaneous red fluorescence in loaded samples; fig. 4 (c) is the confocal laser microscopy of spontaneous green fluorescence of the sample after loading; fig. 4 (d) is the confocal laser microscopy of fig. 4 (a), fig. 4 (b) and fig. 4 (c) after the combination of the three. Observation of confocal laser microscopy showed that the MSNs sample was



successfully loaded with doxorubicin hydrochloride judge the effect of the MSNs samples in the drug loading, the need to obtain the absorbance of different concentration of hydrochloric acid doxorubicin and MSNs samples of the drug, to complete the absorbance of acquisition and drug loadings calculation, the calculation formula by using ultraviolet spectrophotometer and drug loadings, and draw the hydrochloric acid doxorubicin standard curve, the result is shown in

fig. 5 and are shown in Table 2.



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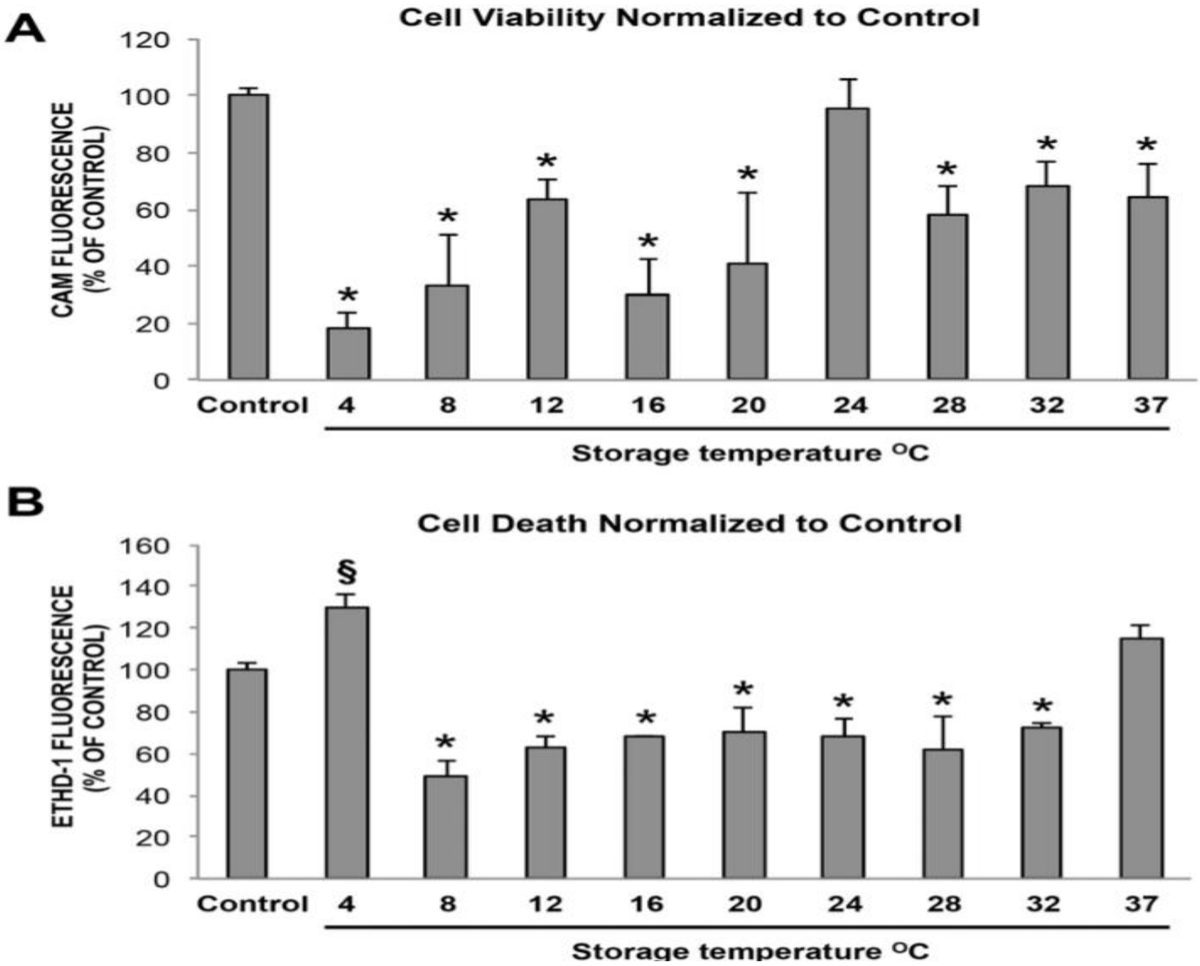
The text presents a formula for calculating drug load in MSN samples. The formula is: Drug loading equals the input drug quality minus the free drug quality, divided by the loading drug quality, and then divided into the input nanomaterial quality, all multiplied by 100 percent.Drug load and encapsulation rate depend heavily on ultrafiltration centrifugation times. Lower centrifugation times prevent separation of free drugs from carriers, increasing drug load and encapsulation. After three rounds of ultrafiltration, MSNs showed 55% drug loading and a 90%+ encapsulation rate for doxorubicin hydrochloride.order to study the effect of different incubation times (24 h, 48 h, 72 h) on the survival rate of gastric cancer cells, the survival rate of five MSNs samples loaded with doxorubicin hydrochloride at different concentrations was calculated by cell survival. Multiple tests were conducted and the survival rate was calculated as the average value for analysis. The error of multiple tests was small and the results were shown in fig. 6.



shown in fig. 6, when the concentration of MSNs loaded with doxorubicin hydrochloride increased from 0.01 mg/ml to 0.09 mg/ml, the inhibitory effect on gastric cancer cells was concentration-dependent and the higher the concentration, the lower the survival rate of gastric cancer cells, indicating that nano-controlled release anticancer drugs can inhibit gastric cancer cells, which can be used as a basis for treatment of gastric cancer.paper, the in vitro performance of nano-controlled release anticancer drugs in gastric cancer cells was studied. Taking silicon dioxide nanoparticles (MSNs) as an example, MSNs samples loaded with doxorubicin hydrochloride at different concentrations were prepared for relevant experiments. The experimental results showed that: the drug release performance of the sample was tested and the results showed that the MSNs sample prepared could promote the drug release in the slightly acidic environment of the tumor site with the gradual increase of acidity of the buffer; the adsorptiondesorption test results and load characterization results were observed through the relevant test of drug load on the samples. It was known that the average mesopores diameter of MSNs samples was about 3 nm, so the loading of doxorubicin hydrochloride was successfully completed; by calculating the drug loading capacity and encapsulation rate of MSNs samples, the results showed that MSNs samples had a certain sustained release effect because of their large drug loading capacity and high encapsulation rate; by monitoring gastric cancer cell survival, judge when MSNs sample concentration is higher, the survival rate of gastric cancer cells is lower, this is because the MSNs having long cycle, can improve the stability of the drug in the body, take the drugs through the high permeability often Direct- Current (DC) effect to the body more tumor site, thus effectively inhibit the growth of gastric cancer cells. The next step is to use the nanodelivery system in vivo for other cancers.

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Conflict of interests:
The authors declared no conflict of interest.

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